Art. XV.—*Bitter Pit and Sensitivity of Apples to Poisons.*

An Answer to Prof. A. J. Ewart.

by

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and

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This opportunity has kindly been given us of replying to a paper by Prof. A. J. Ewart, appearing in the Proceedings of this Society, Vol. XXVI., p. 228, March, 1914, in which he criticises a paper by R. H. Greenwood and A. C. H. Rothera, forming portion of the Second Progress Report, issued by Mr. D. McAlpine.

The position briefly is this:

A theory that Bitter Pit might be due to poisoning of certain cell groups in the apple was put forward by Dr. Jean White, and strenuously supported by Prof. Ewart. This theory included the statement that the apple cells of the areas affected with pit have their diastatic enzymes destroyed—or inhibited—by the poison before the cells themselves are killed. It is logical, if this be so, to attempt to show that bitter pit tissue contains some poison capable of inhibiting diastatic action, and Greenwood and Rothera searched first for such a poison in a soluble form, second in an insoluble form. They were unable to get any evidence of inhibition, their results either showing no effect, or in the case of malt diastase an acceleration with both normal pulp and pitted material. This acceleration was assigned to the beneficial effect which traces of organic acids exert upon malt diastase, which explanation is still held to be the correct one for this result, since it naturally accounts for the slightly greater acceleration produced by healthy pulp cells than by bitter pit, and also for the greater retardation of ptyalin (which is injured by slight acidity), by the former than the latter.

Against these experimental results Prof. Ewart raises various criticisms.

His first is a complete denial. He writes: "On repeating these experiments with filtered solutions of malt diastase dissolved in distilled water, I am able to give them emphatic contradiction. Prolonged contact with pounded apple pulp, boiled or unboiled,

bitter pit, or normal, practically destroys diastase in one to three days."

But in the next paragraph it appears that 20 grms. of pounded pulp were added to only 10 c.c. of 1% *Taka* diastase. No experiments are quoted which are comparable with Greenwood and Rothera's, in which 1 grm. of pounded pulp was used with 5 c.c. of a 5% *malt* diastase, and the emphatic contradiction is therefore most unjustifiable.

The second criticism is that the observed accelerating action on malt diastase was not an acceleration, but only an apparent acceleration, due to an experimental pitfall.

Owing to actions of tannic acid on starch solutions causing precipitation of the starch, and also to the influence of tannic acid upon the starch iodine test employed, Greenwood and Rothera are assumed to have been misled into taking the digestion of the starch as complete, when it really was still incomplete.

Though convinced that a real acceleration of malt diastase had been obtained with both normal apple tissue and bitter pit material, and that the experimental technique employed excluded the pitfalls suggested by Prof. Ewart, it was decided to carefully investigate the criticism raised as to the action of tannic acid.

Freely acknowledging full indebtedness to Professor Ewart for bringing the tannic acid complications to our notice, we find that such complications cannot be applied to refute the experimental results of Greenwood and Rothera.

In the first place, though the blue colour produced by starch with a small amount of iodine can be destroyed by tannic acid, the proportions of the reagents are quite different to those of the experiments of Greenwood and Rothera, in which the maximum tannic acid could not exceed .002% final concentration, and in which the iodine was always used in large excess.

For, in following a starch digestion, it is customary to remove 1 drop of the starch solution, which is then mixed with 1 drop of a 1% iodine solution, and the blue colour produced under such conditions is uninfluenced by tannic acid in concentrations up to 1%.

Only when the quantity of tannic acid is large, and the iodine very little in amount, does the decolourising power of the tannic acid become important.

Then again, we find that Ewart is unfortunate in his second point that tannic acid inhibits diastatic action by forming a compound with starch which is resistant to the ferment.

Although tannic acid (in the concentrations with which we are concerned, when dealing with apple tissue or juice) does produce a slight cloudiness in a 1% filtered starch solution, this does not render the starch any less readily digested by diastase. With the weaker tannic acid solutions (as Prof. Ewart points out) the cloudiness vanishes at 35° C.

In fact, with the taka diastase (Merck) in the possession of the laboratory, the presence of small amounts of tannic acid caused an acceleration of the enzyme action, and a slowing was only obtained when the tannic acid concentration was approximately five times as great as in apple juice.

It proved a most fortunate circumstance for us that the taka diastase preparation in our possession (the same as that used by Greenwood and Rothera) showed this acceleration, for it permitted us to show that (at least for the tannic acid concentrations up to .25%) tannic acid does not form a starch compound of less digestibility, and that where it does inhibit diastatic action it does so by precipitating the enzyme as suggested by Payen.[1]

Solutions of the taka diastase in our possession gave no immediate precipitate with dilute tannic acid, but in some of our experiments, in which a weak enzyme solution was used, a precipitate did form after two hours, and was accompanied by a slowing of the rate of action.

This slowing apparently depended upon the flocculation of the enzyme per se, and was not proportional to the amount of tannic acid present. In fact, a slightly greater amount of tannic acid produced less slowing, probably because the accelerating factor was present simultaneously.

Careful tests were made in order to be certain that the tannic acid acceleration was not due to an influence exerted upon the starch iodine colour.

Greenwood and Rothera used strong solutions of taka diastase, and in the light of present results the very small amount of tannic acid extracted from healthy apple pulp, or pitted cells, could only have had a slight accelerating influence.

With the malt diastase (Merck) (the same preparation as that used by Greenwood and Rothera) tannic acid solutions, even when dilute, always gave a precipitate, with solutions of the enzyme, accompanied by a retardation of action. In Greenwood and Rothera's experiments with malt diastase, accelerations were

1. Quoted from Czapec Biochemie der Pflanzen, vol. i., p. 345.

obtained, so that the invoking of the tannic acid retardation is not applicable.

We therefore maintain (i) that Ewart's tannic acid complications are not applicable to the experiments of Greenwood and Rothera; (ii) that Ewart is incorrect in assigning the tannic acid retardation to an action upon the starch; and (iii) that experimental results obtained under quite different conditions and with quite different proportions of reagents have been used in a wholly unjustifiable manner, as though applicable to Greenwood and Rothera's conditions, and reagent concentrations.

1.—Action of Tannic Acid on Filtered Starch Solutions.

1% tannic acid solution was added to 1% starch solution in the proportions of 1:1; 1:3; 1:7; and 1:15 respectively, the final concoctions of tannic acid being 0.5%; 0.25%; 0.125%, and 0.0625% respectively. They were mixed and kept at room temperature.

The first two gave a cloudiness immediately, which became dense on standing; but after eighteen hours there was no sign of a precipitate.

The third gave a very slight cloudiness on first mixing, but this became more marked on standing.

The fourth showed no appreciable change from a control to which an amount of water equal to the tannic acid solution had been added, but after standing eighteen hours there was a just perceptible difference.

In the course of one of the experiments, to be described later, 1% tannic acid and 1% filtered starch solutions were mixed, so that the final concentrations were .2% tannic acid, and .8% starch respectively. The starch solution had been made up five days previously, and had not been filtered in the meantime.

A cloudiness developed immediately on mixing, which was done at room temperature.

This was then placed in a water bath kept at 38°—40°C., with a control. At the temperature of the bath it became much clearer, being but little denser than the control (which contained .8% starch solution), and at the end of twenty-three hours there was a slight transparent precipitate at the bottom of both tubes, being slightly greater in that containing the tannic acid.

2.—The Action of Tannic Acid on the iodine test for dextrins formed during diastatic action on starch.

A final concentration of 1% tannic acid was found to have no effect on this test, as used in Greenwood and Rothera's, and the following experiments. This is because the conditions involve an excess of iodine. The interference of tannic acid with the starch and dextrin colours is due apparently to its forming a combination with the iodine, and naturally ceases when the iodine is in excess.

3.—Action of Tannic Acid on the diastatic hydrolyses of starch.

Filtered starch solution was used throughout the experiments, which were done at a temperature of 38°—40°C.

Experiment 1. (Tubes each contained 15 c.c. of mixture.)

Using a 2.5% solution of taka diastase, which proved rapid in action, the following mixtures were made up, and tested for comparative rates of action.

	Starch (1%).	Diastase (2.5%).	Tannic Acid (final concentration).
a (control)	10 c.c.	1 c.c.	0
b	10 c.c.	1 c.c.	.5%
c	10 c.c.	1 c.c.	.25%
d	10 c.c.	1 c.c.	.125%

c and *d* were finished in about 5½ minutes, and *a* about 2 minutes later. *b* still gave a strong red-brown at the end of 20 minutes.

Experiment 2. (Tubes each contained 10 c.c. of mixture.)

This was done, using taka diastase, with weaker concoctions of tannic acid than those used in Experiment 1.

	Starch (1%).	Diastase (2.5%).	Tannic Acid (final concentration).
a (control)	8 c.c.	1 c.c.	0
b	8 c.c.	1 c.c.	.1%
c	8 c.c.	1 c.c.	.05%
d	8 c.c.	1 c c.	.025%

In 2 minutes, the control *a* still gave a red-violet, whilst the others were all red. *b* was finished in 5 minutes, *c* and *d* in about 8 minutes, and control *a* in 12 minutes.

Experiment 3. (Tubes each contained 10 c.c. of mixture.)
Using malt diastase.

	Starch (1%).	Diastase (2.5%).	Tannic Acid (final concentration).
a (control)	8 c.c.	1 c.c.	0
b	8 c.c.	1 c.c.	.1%
c	8 c.c.	1 c.c.	.05%
d	8 c.c.	1 c.c.	.025%

Although the acid and starch solutions were mixed before adding to the diastase, the concentrations of tannic acid were strong enough to cause a precipitate to form in the diastase solution. (This was *not* the case with taka diastase in Experiment 1.)

In 12 minutes the control *a* was well into the red, while the others were all blue. In 20 minutes *d* was beginning to show traces of violet, the others being still blue.

The effect on saliva was parallel, a precipitate being formed by .025% of tannic acid.

Experiment 4. (Tubes each contained 10 c.c. of mixture.)

This was done with weak taka diastase.

	Starch (1%).	Diastase (.25%).	Tannic Acid.
a (control)	8 c.c.	1 c.c.	0
b	8 c.c.	1 c.c.	.3%
c	8 c.c.	1 c.c.	.15%
d	8 c.c.	1 c.c.	.1%

These were left in a water bath overnight. The temperature started at 40°C., but fell to 36°C. during the night.

On testing, after 18½ hours, the control *a* had finished, *d* gave a very slight colour, *c* gave more colour (red), whilst *b* gave a very red brown.

In this experiment, concentrations of tannic acid, which gave an acceleration in the short experiments, here gave a very definite retardation.

The following experiments were done to determine whether this retardation was due to action on the ferment or on the starch.

Experiment 5. (Tubes each contained 10 c.c. of mixture.)

	Taka Diastase (2.5%).	Tannic Acid (final concentration).
a	8 c.c.	.2%
b	8 c.c.	.05%
c (control)	8 c.c.	0

These were kept in a water bath at 38°—40°C.

In 2 hours a fine, dispersed coagulum had appeared in about equal amounts in *a* and *b*.

This had slightly increased and settled out at the end of 23 hours, and by this time a slight precipitate had appeared in the control *c*. *a* and *b* were also darker in colour than *c*.

a, *b*, and *c* were shaken thoroughly, and 1 c.c of each was tested on 5 c.c. of starch solution (1%).

The control finished in $2\frac{1}{2}$ minutes, *a* in 4 minutes, and *b* in $5\frac{1}{2}$ minutes.

Although *a* had been in contact with a greater percentage of tannic acid, yet its action was quicker than that of *b*.

This may be explained by supposing that the formation of a coagulum was the sole inhibiting factor, and that the acceleration was caused by the presence of .04% of tannic acid in the final starch-diastase mixture, in the case of *a*, whereas only .01% was present when testing *b*. [No acid being present in control.]

Experiment 6. (Tubes each contained 10 c.c. of mixture.)

	Starch (1%).	Tannic Acid (final concentration).
a	- 8 c.c.	- .2%
b	- 8 c.c.	- .05%
c (control)	- 8 c.c.	- 0

In testing these, it was obviously necessary to compare them with the control in the presence of a corresponding amount of acid, hence the control was divided into two.

The following tubes were made up (each containing 5 c.c. of mixture).

		Starch.	Diastase.	Tannic Acid.	H_2O.
a	1	3 c.c. of *a*	1 c.c. 1% Taka diastase	0	1 c.c.
	2	3 c.c. of *c* (control)	,,	.6 c.c. of 1%	.4 c.c.
b	3	3 c.c. of *b*	,,	0	1 c.c.
	4	3 c.c. of *c* (control)	,,	.15 c.c. of 1%	.85 c.c.

1 and *2* then contained .12% of Tannic Acid
and *3* and *4* ,, ,, .03% ,, ,,

It was found that *1* and *2* went neck and neck (6 minutes), and at a faster rate than *3* and *4*, which also went neck and neck (10 minutes).

This experiment shows that the prolonged action of .2% tannic acid on .8% *filtered* starch solution does not materially affect its subsequent hydrolyses by taka diastase.